Association of 11-hydroxysteroid dehydrogenase type I expression and activity with estrogen receptor in adipose tissue from postmenopausal women

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Association of 11β-Hydroxysteroid Dehydrogenase Type 1 Expression and Activity with Estrogen Receptor β in Adipose Tissue from Postmenopausal Women

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Abstract

Objective—11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) regenerates active cortisol from inert cortisone in adipose tissue. Elevated adipose tissue 11βHSD1 activity is observed in obese humans and rodents where it is linked to obesity and its metabolic consequences. Menopause is also associated with increased abdominal fat accumulation suggesting that estrogen is also important in adipose tissue metabolism. The purpose of this current study was to establish whether estrogen signalling through estrogen receptors-α and -β (ERα and ERβ) can influence 11βHSD1 in premenopausal and postmenopausal adipose tissue.

Methods—19 premenopausal (aged 26±5, BMI 23.6±1.6) and 23 postmenopausal healthy women (aged 63±4, BMI 23.4±1.9) were studied. Subcutaneous adipose tissue biopsies and fasting venous blood samples were taken. Body composition was measured by bio-electrical impedance analysis. Human SGBS adipocyte cells were treated with ERα and ERβ-specific agonists for 24h. Basic anthropometric data, Serum 17β-estradiol and progesterone concentrations, ERα and ERβ mRNA levels and 11βHSD1 mRNA, protein and activity levels were assessed.

Results—ERβ and 11βHSD1, but not ERα mRNA was significantly increased in adipose tissue from postmenopausal women compared to premenopausal women. ERβ had a significant positive correlation with the mRNA level of 11βHSD1 in adipose tissue from pre- and postmenopausal women. This association between ERβ and 11βHSD1 was greatest in adipose tissue from postmenopausal women. In human SGBS adipocytes, diarylpropiolnitrile (DPN), a selective ERβ agonist increased 11βHSD1 mRNA, protein and activity levels.

Conclusions—We conclude that in adipose tissue, ERβ-mediated estrogen-signalling can upregulate 11βHSD1 and that this may be of particular importance in postmenopausal women.

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Keywords

menopause; Estrogen receptor β; 11β-Hydroxysteroid Dehydrogenase Type 1; adipose tissue

Introduction

The onset of menopause is associated with an accumulation of adipose tissue[1] and this may contribute to the increased metabolic and cardiovascular risk seen in postmenopausal women. Chronic glucocorticoid excess (e.g. in Cushing's syndrome) also causes obesity and its associated metabolic dysfunction. Whilst plasma cortisol levels are not elevated in obesity[2], recent evidence suggests that there is a selective increase in glucocorticoid regeneration in adipose tissue[3]. Specifically, the microsomal enzyme 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) which catalyses the intracellular reactivation of cortisol from inert cortisone, is selectively increased in adipose tissue in obese humans and in rodent models of obesity[4;5]. This appears to be of pathophysiological importance since mice engineered to selectively over-express 11βHSD1 in adipose tissue develop visceral obesity and metabolic syndrome[6], whereas mice lacking 11βHSD1 resist glucose intolerance, insulin resistance and hyperlipidemia on high-fat diet[7]. In humans, 11βHSD1 inhibition has analogous effects[8].

Estrogen signalling is predominantly mediated via the two nuclear estrogen receptors α and β (ERα and ERβ), both of which are present in human adipose tissue[9]. Human studies on sex steroid control of 11βHSD1 in adipose tissue are scarce and inconclusive; reporting both up-regulation and no effect of estrogen, using a variety of doses mainly *in vitro*[10]. Here we have explored the possibility of a functional link between ERα/β signalling and 11βHSD1 expression/activity in adipose tissue from premenopausal and postmenopausal women.

Methods

Experiments in Humans

All women gave written, informed consent and all clinical investigation was conducted according to the principles expressed in the Declaration of Helsinki. The study of pre- and postmenopausal women was approved by the Ethical Committee for human research at Umeå University, Umeå, Sweden, approval ID 03-339. 19 premenopausal and 23 postmenopausal, healthy, normal weight women were recruited by advertisements in the local newspapers and within the Umeå University Hospital and campus areas. Exclusion criteria were: diabetes, thyroid dysfunction, hepatic and renal disease, use of tobacco, hormonal contraceptives, systemic gonadal hormone replacement therapy, or oral glucocorticoid medication. None of the postmenopausal women reported menstrual periods within the last 12 months. One premenopausal woman used inhaled steroids for asthma (budesonide, 400 μg/24 h). Three postmenopausal women had well-controlled hypertension treated with β-blockers, diuretics, or calcium antagonist, one took tolterodine for urinary incontinence and bisphosphonates for osteoporosis, and two used topical E2 or estriol treatment. Further details of the participants included in this study have been described previously[11].

Clinical protocol—Premenopausal women were evaluated during the follicular phase of the menstrual cycle to negate the possible effects of hormonal fluctuations. Menstrual phase or postmenopausal status was confirmed by measuring serum 17β-estradiol and progesterone levels (described below).

Anthropometric measurements and adipose tissue biopsies were performed on separate days. Weight to the nearest 0.1 kg (with subjects wearing light clothes) and height and waist circumference to the nearest 0.5 cm was measured. Body composition was measured by bio-electrical impedance analysis using a Bodystat 1500 (Bodystat Ltd., Isle of Man, British Isles). Approximately 2 g of periumbilical superficial subcutaneous adipose tissue was excised under local anaesthesia with lidocaine (Xylocaine® without adrenaline, AstraZeneca, Sweden) after an overnight fast. Tissue was snap frozen in liquid nitrogen within 5 minutes after removal, and stored at −80°C until further analyses.

Venous blood samples for routine laboratory tests were drawn at the time of anthropometric measurements. Venous blood samples for serum analyses (described below) were drawn in the mornings of the adipose tissue biopsies after at least eight hours of fasting.

Laboratory methods

Serum Analyses—17β-Estradiol, progesterone and cortisol were measured in samples drawn at the time of the biopsy. Serum cortisol and progesterone were analyzed by electrochemiluminescence immunoassays, on a Modular Analytics E170 (all from Roche AB, Stockholm, Sweden). 17β-Estradiol was measured using an ultra sensitive radioimmunoassay (ESTR-US-CT, CIS bio international, Gif-sur-Yvette, Cedex, France) (intra- and interassay coefficients of variation (CV); 2.8–18.1% and 5.8–17.6%, respectively).

Cell culture—Human SGBS (Simpson-Golabi-Behmel Syndrome) adipocyte cells which have previously been shown to express both ERα and ERβ[12] were maintained and differentiated into adipocytes, as previously described[13]. For experimental manipulations, cells were serum-starved overnight in phenol-red-free medium containing 0.1% BSA. After serum starvation, cells were treated with diarylpropiolnitrile (DPN) or propyl-pyrazole triol (PPT), at the concentrations indicated for 24 h. All experiments were performed in triplicate and repeated three times.

Quantification of mRNA—Total RNA was extracted from snap-frozen adipose tissue or SGBS adipocytes according to the manufacturer's protocol using the RNeasy® lipid tissue midi kit (Qiagen Nordic, Qiagen House, West Sussex, UK). RNA concentrations were measured on a ND-1000 Spectrophotometer (NanoDrop Technologies, Bancroft Building, Wilmington, DE, USA) and integrity was evaluated on a 1% agarose electrophoretic gel and visualized with ethidium bromide under UV-light.

One microgram of RNA was reverse transcribed into cDNA using TaqMan® Reverse Transcription Reagents (Roche Molecular Systems, Inc., Branchburg, NJ, USA). cDNA was incubated in triplicate with 1x gene-specific assay mix (Applied Biosystems, Warrington, UK) in 1x Universal PCR Master Mix (Roche). PCR cycling and detection of fluorescent signal was carried out on an ABI Prism® 7000 Sequence Detection System (Applied Biosystems). Triplicates were deemed acceptable if the standard deviation of the crossing point was < 0.5 cycles. A standard curve (y axis crossing point, x axis log concentration) for each gene was generated by serial dilution of cDNAs pooled from different samples and fitted with a straight line and deemed acceptable if reaction efficiency was between 1.7 and 2.1. For SGBS cells, results were corrected for with TATA box binding protein (TBP) and for human adipose tissue, results were corrected for cyclophilin A which had the lowest coefficients of variation and the best stability value, based on the Normfinder algorithm (http://www.mdl.dk/publicationsnormfinder.htm) out of three tested endogenous controls.

The prevalidated assays used were: human ERα, Hs00174860_m1; human ERβ, Hs00230957_m1; human 11βHSD1, Hs01547870_m1; and the endogenous controls

cyclophilin A (PPIA), Hs99999904_m1 and TBP Hs00427620_m1; (all from Applied Biosystems).

Western blot—SGBS cells were washed in ice-cold PBS and lysates prepared as described previously[13]. 50μg of protein were diluted in sample buffer containing DTT, denatured and run on 12% polyacrylamide gels, and transferred to nitrocellulose for Western blotting. Membranes were probed with 11βHSD1 antibody (The Binding Site Group Ltd, Birmingham, UK). Protein loaded was corrected for using a monoclonal antibody against β-tubulin (Millipore, Watford, UK). Proteins were visualised with an Alexa Fluor® secondary antibody (Li-cor Biosciences, Cambridge, UK) and band intensities were quantified using the Odyssey infrared imaging system (Li-cor Biosciences).

Enzymology—11βHSD1 activity was measured in the 11β-reductase direction in intact differentiated SGBS adipocytes. 3×10^5 cells/ well in 6-well plates were differentiated as described above. Each well was incubated at 37°C in 2ml of serum - and phenol-red-free medium containing 1pM [^{3}H]-cortisone (GE Healthcare; Aylesbury, UK) and 1μM cortisone (Sigma-Aldrich Company Ltd, Dorset, UK). All incubations were performed in triplicate for 24h. Assay conditions were optimized to ensure first-order kinetics. After incubation steroids were extracted from 2ml of medium with Sep-pak columns (C18, 0.3g, Waters, Milford, MA, USA). The organic phase was evaporated under nitrogen and extracts were re-suspended in mobile phase (60% water, 10% acetonitrile, 30% methanol). Percentage recovery was 90 ± 2.3% for cortisone and 92 ± 3.1% for cortisol. Steroids were separated by HPLC (Waters) and compared to known tritiated standards (GE Healthcare) using a C_{18} Reverse-phase column (Sunfire C_{18},15cm,id 2.6mm, pore size 5μm; Waters) and were quantified by on-line liquid scintillation counting.

Statistical analyses

Data are presented as mean ± SEM, unless stated otherwise, and were natural log-transformed when necessary to achieve parametric distributions. Unpaired Student's t-tests were used to compare means between groups. Associations between parameters were assessed using Pearson correlation (r). Partial correlation analysis was used in all groups to adjust for obesity measures. Statistical calculations were performed using the SPSS software (release 14.0.1, SPSS Inc., 233 S. Wacker Drive, Chicago, IL). $P<0.05$ was considered statistically significant.

Results

Participant characteristics

The percentage of body fat and waist-hip-ratio was significantly higher in postmenopausal women compared to premenopausal women (Table 1). Serum E2 and progesterone levels were significantly lower in postmenopausal vs. premenopausal women regardless of menstrual phase (Table 1). Serum cortisol levels were not different between pre- and postmenopausal women. Notably, there was a high variability regarding ERβ and 11βHSD1 expression in the postmenopausal study group.

ERβ and 11βHSD1 mRNA levels are increased in adipose tissue from postmenopausal women

There was no difference in the level of ERα mRNA between pre- and postmenopausal women, (Figure 1A). The expression level of ERβ and 11βHSD1 mRNA was two-fold higher in postmenopausal women compared to premenopausal women in follicular phase (Figure 1A).

11βHSD1 mRNA is correlated with ERβ but not ERα mRNA in adipose tissue

A strong positive correlation between 11βHSD1 and ERβ mRNA levels was observed in subcutaneous adipose tissue irrespective of menopausal status (Figure 1B). These associations remained after adjustments for obesity measures, (BMI, waist and fat percentage). In contrast, no association between ERα and 11βHSD1 expression was found (Figure 1C).

Adipocyte 11βHSD1 expression and activity is up-regulated by an ERβ-agonist *in vitro*

The ERβ-specific agonist, DPN, up-regulated 11βHSD1 mRNA in human SGBS adipocytes (Figure 2A). In contrast, the ERα-specific agonist PPT did not have any effect on 11βHSD1 mRNA levels (Figure 2A). 11βHSD1 protein expression (Figure 2B) and activity (Figure 2C) were also increased by DPN.

Discussion

These results identify a potential mechanism by which estrogens acting via ERβ may enhance local glucocorticoid action in adipose tissue of postmenopausal women, involving up-regulation of adipose 11βHSD1 expression. This association between ERβ and 11βHSD1 was greatest in adipose tissue from postmenopausal women. Crucially, a direct effect of selectively activating ERβ on adipocyte 11βHSD1 expression and activity was demonstrated *in vitro*.

In postmenopausal women, where ovarian steroid biosynthesis has ceased, the major source of estrogens is local aromatase expression in the adipose tissue, the function of which increases with age[14]. We previously reported a positive association between aromatase and 11βHSD1 gene expression in adipose tissue suggesting an up-regulatory effect of local estrogen production on 11βHSD1 expression in adipose tissue[11]. Estrogen signalling is predominantly mediated via the two nuclear receptors ERα and ERβ, both of which are present in human adipose tissue[9]. Here, we have shown that ERβ mRNA levels are two-fold higher in subcutaneous adipose tissue from postmenopausal women compared to premenopausal women and that expression of this receptor is strongly correlated with adipose expression of 11βHSD1. Consequently, in postmenopausal women, adipose 11βHSD1 may be increased by local estrogens acting via ERβ, resulting in increased levels of active glucocorticoids in adipose tissue. Since increased glucocorticoid activity in adipose tissue is associated with obesity[4], this mechanism may contribute to the increase in body fat observed in our cohort of postmenopausal women. Furthermore, aromatase promoter activity is driven by glucocorticoids hence increased 11βHSD1 activity in adipose tissue will further amplify this process. The reasons for the high variability in ERβ and 11βHSD1 gene expression among postmenopausal women is not clear and needs further studies regarding putative regulatory factors including a possible interaction with aromatase activity.

Despite a significant increase in percentage fat mass and waist-hip ratio in the postmenopausal women the BMI of both groups of women were in the normal range probably reflecting a decrease in lean mass and bone mineral density which is commonly associated with menopause[15]. 11βHSD1 activity has been shown to be increased in adipose tissue of even moderately obese women[4] On the other hand it has been reported that there is no significant correlation between the respective levels of ERα or ERβ in subcutaneous or omental adipose tissue with obesity[16]. We have had an opportunity to assess this correlation in subcutaneous and visceral adipose tissue of obese premenopausal women and there remains a strong association between ERβ and 11βHSD1 in both depots (unpublished results). Therefore, we would predict that the correlation would still remain between ERβ

and 11βHSD1 in obese women. Unfortunately we did not have the opportunity to assess whether there is an association between ERβ and 11βHSD1 expression in visceral adipose tissue in this cohort of women. This would have been interesting since redistribution of adipose tissue to abdominal visceral depots is commonly observed with menopause. Several human studies have shown higher expression of 11βHSD1 in the subcutaneous depot than other depots. Furthermore, the subcutaneous depot appears to be more predictive for obesity and metabolic parameters[17]. Importantly, subcutaneous 11βHSD1 correlates with measures of central fat accumulation and is an independent predictor for central fat accumulation[17], emphasizing the important role of the 11βHSD1 enzyme in subcutaneous adipose tissue.

This observation of a pro-adipogenic role for ERβ is supported by studies in knock-out mice. ERα knock-out (αERKO) and double knock-out (α/βERKO) mice show marked obesity[18;19] whereas ERβ knock-out (βERKO) mice are normal weight. However, αERKO mice also have elevated 17β-estradiol levels thus increasing ERβ signalling[20] and when ERβ signalling is removed in αERKO by ovariectomy, the fat-pad weights and adipocyte size decrease[20], suggesting adipogenic effects of ERβ signalling. Furthermore, in humans, Shin and colleagues reported that subjects with more ERβ than ERα in omental adipose tissue had a greater degree of adiposity[16].

In vitro, the ERβ agonist, DPN had a maximal effect on 11βHSD1 at 1-10nM. Since DPN has its highest specificity for ERβ at these concentrations[21], these results support our conclusion that activation of ERβ can directly up-regulate 11βHSD1 in adipocytes. These findings are also supported by previous data showing that 17β-estradiol can activate the 11βHSD1 promoter in transfected adipocytes[22].

Conclusion

In conclusion, this study implies a link between adipose ERβ signalling and local glucocorticoid metabolism that may promote adipogenesis. This is particularly relevant in the postmenopausal setting where the beneficial effects of activating ERα are diminished due to a decrease in circulating 17β-estradiol levels and may contribute to the increase in obesity observed in postmenopausal women.

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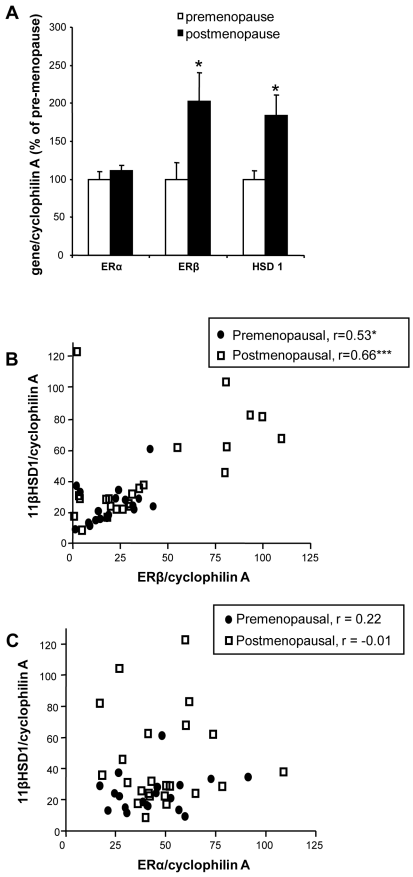

Figure 1. A strong positive correlation between 11βHSD1 and ERβ in adipose tissue

(A) ERα, ERβ and 11βHSD1 mRNA expression in SAT from premenopausal women in follicular phase of the menstrual cycle compared to postmenopausal women. Data are mean ± standard error, n=19 for premenopausal women and n=23 for postmenopausal women. **P<0.05 vs* premenopause. **(B)** Correlations between ERβ and 11βHSD1 in SAT from pre- and postmenopausal women (n=19 for premenopausal women and n=23 for postmenopausal women). Closed circles represent premenopausal women and open squares represent postmenopausal women where $r = 0.53$, $P < 0.05$ for premenopausal women and $r = 0.66$, $P < 0.0001$ for postmenopaisal women. **(C)** Correlations between ERα and 11βHSD1 in SAT from pre- and postmenopausal women (n=19 for premenopausal women and n=23 for postmenopausal women). Closed circles represent premenopausal women and open squares represent postmenopausal women where $r = 0.22$ for premenopausal women and $r = -0.01$ for postmenopaisal women. *SAT, subcutaneous adipose tissue; ER, estrogen receptor ; r , Pearson correlation coefficient; 11βHSD1, 11β-Hydroxysteroid dehydrogenase type 1;*

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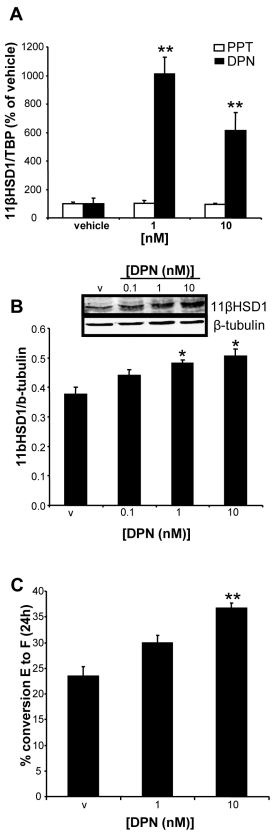

Figure 2. 11βHSD1 expression and activity is up-regulated by an ERβ-specific agonist *in vitro*

(A) 11βHSD1 gene expression in SGBS cells after incubation with the ERβ-specific agonist DPN. Data are mean ± standard error, n=3 triplicates. **P<0.01 *vs* vehicle. **(B)** Representative Western blot of 11βHSD1 protein expression normalized against β-tubulin, Data are mean ± standard error, n=3 triplicates. *P<0.05 *vs* vehicle (v) **(C)** 11βHSD1 enzyme activity in intact cells after DPN stimulation measured as % conversion of cortisone (E) to cortisol (F) after 24h incubation. Data are mean ± standard error. n=3 triplicates. **P<0.01 *vs* vehicle (v). *ER, estrogen receptor ; 11βHSD1, 11β-Hydroxysteroid dehydrogenase type 1; DPN, diarylpropionitrile; SGBS, Simpson-Golabi-Behmel Syndrome.*

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TABLE 1

Participant characteristics

	Premenopausal (n = 19)	Postmenopausal (n = 23)
Age, y	26 ± 5	63 ± 4[a]
Body fat, %	27.4 ± 3.4	36.0 ± 5.3[a]
Body mass index, kg/m^2	23.6 ± 1.6	23.4 ± 1.9
Waist circumference, cm	80.1 ± 6.8	82.8 ± 5.5
Waist-hip ratio	0.80 ± 0.06	0.84 ± 0.05[b]
Cortisol, nM	476 ± 184	472 ± 141
Estradiol, pM	201 ± 142	19.6 ± 6.3[a]
Progesterone, nM	2.30 ± 0.79	1.32 ± 0.47[a]

Data are expressed as mean ± SD.

[a] $P < 0.001$ versus premenopausal women.